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RESULTS OF REPEATABILITY AND STANDARDIZATION TESTS IN CATTLE BLOOD TYPING

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A rapid accumulation of new knowledge has occurred in the field of cattle blood typing in the past two decades. Several recent reviews and articles describe the progress and application of the findings (1-7). Along with the progress, however, came the problem of coordination among the workers in this field. As new blood factors and phenogroups were discovered it became increasingly difficult to standardize nomenclature and classification in the different blood-group systems. Newer laboratories were particularly concerned since they needed to compare the blood-typing reagents which they developed with those used by others to recognize the established blood factors.

In an effort to facilitate standardization and estimate repeatability in cattle blood typing, the Dairy Cattle Research Branch instituted a cooperative program of comparative testing in March 1956. Aliquots of blood samples from 40 animals were sent to the participating laboratories. Various breeds of cattle were used with emphasis on family groups to aid genetic studies. The aliquots were then tested concurrently by the laboratories and the results were reported to the Dairy Cattle Research Branch where they were duplicated and sent to all participating laboratories. Thus, each laboratory had the opportunity to compare its findings with those of others that had tested blood from the same animals.

Six trials in this program have been completed. Over 75,000 individual tests of cattle red blood cells were made. Laboratories in Canada, Denmark, Finland, Holland, Norway, South Africa, Sweden, the United States (California, Ohio, Wisconsin and Wyoming) and West Germany (Göttingen and Munich) have participated at least once. Table 1 shows the dates of the trials and the laboratories participating. Laboratories were given letter-codes for the purpose of this report.

The program is most valuable to the extent that it allows comparison of blood-typing reagents produced in different laboratories. Also important is the opportunity for coordination among workers in the task of phenogrouping which is the most significant aspect of blood grouping from the genetic standpoint. The program has also provided data for a study of repeatability in cattle blood typing. The data permitted estimation of two items of interest. One was the extent to which the laboratories agreed with themselves when they unknowingly tested blood from the same animals twice. Some samples were repeated on a within-trial basis as well as between trials. In the case of within-trial repeat samples each laboratory received two tubes of blood from the same animal without being told that they were duplicates. For the between-trial repeats certain animals were used as donors in two different trials. Each time a laboratory tested repeat samples with a particular reagent the same result was expected. However, if the test was positive in one case and negative in the other then a disagreement was recorded. The number of actual agreements divided by the number of possible agreements constituted the per cent of agreement.

The second estimate that could be made from the data was the agreement among the laboratories when they tested the same cells with the same reagents (reagents produced independently and presumed to detect the same antigenic factors). The reports of reactions were studied within reagents and trials. For example, in a particular trial, if some or all of the participants used a reagent that was supposed to be specific for the same blood factor then the results with that reagent were compared to determine the number of samples of the 40 on which all laboratories agreed. The cells either had the blood factor (positive reaction) or did not (negative reaction). An agreement was recorded when all the laboratories testing a particular sample reported the same result.

Results and Discussion

The results of the analysis of the repeatability data are shown in Table 2. It is obvious that repeatability was very high. Explanation is necessary in the case of laboratory G. It is obvious from the table that G experienced a great deal of difficulty. It seemed, therefore, that the overall means might be considered more representative with the data from G excluded. Actually the overall per cent agreement figures of 97.8 (with G) and 99.0 (without G) differ little. Their magnitude emphasizes the very high repeatability of these tests within laboratories.

The results for the agreement among laboratories are shown in Table 3. Per cent of agreement figures are given in two ways. In the left column the per cent agreement was calculated using all available data. In the right column, the per cent agreement was calculated after omitting the results from the laboratory which, for that particular reagent in a particular trial, had caused the most disagreement. For example, if six laboratories used a reagent designated as A in a particular trial, the per cent of agreement was calculated as described in the second paragraph on page 2. After this had been done, the results from the laboratory causing the most disagreement were omitted, and the per cent of agreement among the remaining five was calculated. The per cent of agreement figures for each reagent were then weighted for each trial according to the number of laboratories using the reagent in that trial and averaged over all trials. These averages are the figures reported in Table 3. In these calculations, laboratory G caused 52.4 per cent of the disagreements. The disagreements caused by the other laboratories accounted for the remaining 47.6 per cent. The differences between the two percentages indicate that frequently one laboratory was responsible for most of the disagreement. This is neither unexpected nor disturbing. Since the main purpose of these trials is to allow comparison of independently developed reagents, it follows that perfect agreement would not be expected. As a matter of fact, the overall means are remarkably high (82.1 per cent with all laboratories and 94.6 per cent with all but one). This is especially notable considering that the overall per cent agreement is 89.0 when only laboratory G's results are excluded. The reagents in Table 3 are listed in decreasing order of agreement. This tends to emphasize differences in the extent to which the reagents are standardized among laboratories. Consideration should be given, however, to the numbers involved in the means. Table 3 also illustrates the results of comparing independently developed new reagents. As an example, the fourth reagent listed in Table 3 is now known as Y' by agreement of the blood-group workers. It was previously known as NF4 and E_1, and these reagents were found to be the same in these comparative tests. This is just one example of the findings in this very important part of the program. Table 4 is similar to Table 3 and summarizes results according to the blood-group systems.

The results of this analysis indicate the high degree of success achieved by workers in cattle blood grouping as a result of their efforts to standardize procedures. The results are especially noteworthy when the sources of error involved are considered. One source of error is in transcription of results. The present program requires a great deal of transcription and some transcription errors undoubtedly are included. In fact, some of these errors were

discovered and pointed out by the participants. However, no corrections were made for them in this analysis since they were considered a part of the overall error being estimated. Occasionally the blood arrived at the laboratories in poor condition due to delay en route. This was particularly true in two instances. However, the results were included since there was no objective way to pick and choose among them. There is no doubt, however, that the tests were not as accurate as they would have been if some of the samples had not deteriorated. The complex hemolytic test itself is a source of error since it involves thousands of small tubes to which the red blood cells, reagents and complement are added a drop at a time. Still another source of error - and the main reason why these comparison tests are so useful to any one of the blood-group workers - is the limited choice of animals, and especially breeds, which is available to most of the workers for production and standardization of reagents. These results indicate the great accuracy with which blood types are regularly determined in cattle when the tests are made by experienced personnel. The results also emphasize the permanence of blood types as a mark of identification since some of the repeat samples were tested at intervals of one or two years.

It is apparent that the opportunity for comparative testing provided by the present program is useful. The tests facilitate standardization and serve as a means of maintaining high accuracy in cattle blood typing. The program is continuing with tests on an annual basis.

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TABLE I

PARTICIPATION OF THE VARIOUS LABORATORIES IN THE REPEATABILITY AND STANDARDIZATION TESTS

Laboratories	March 1956 1	November 1956 2	June 1957 3	January 1958 4	February 1959 5	February 1960 6
A	X	X	X	X	X	X
B	X					
C	X	X				X
D		X	X	X	X	X
E		X	X	X	X	X
F		X	X	X	X	X
G		X	X	X		
H			X	X	X	
I			X	X	X	X
J					X	X
K					X	X
L						X
M						X

TABLE 2

Summary of Results of Tests on Repeat Samples [1] by Laboratory

Laboratory	Between Trial Repeats [2]: No. of Animals	No. of possible agreements [4]	Actual No. of agreements	Percent of agreements	Within Trial Repeats [3]: No. of Animals	No. of possible agreements	Actual No. of agreements	Percent of agreements	All Repeats: Percent agreement (weighted mean) [5]
A	26	1398	1388	99.3	15	768	768	100.0	99.5
B	0	0	0	-	3	54	52	96.3	-
C	7	168	167	99.4	6	156	156	100.0	99.7
D	13	480	473	98.5	12	486	484	99.6	99.1
E	13	494	483	97.8	12	468	468	100.0	98.9
F	13	538	522	97.0	12	528	526	99.6	98.3
G	11	273	213	78.0	9	249	219	88.0	82.8
H	5	296	292	98.6	9	549	549	100.0	99.5
I	5	181	168	92.8	9	405	403	99.5	97.4
J	0	0	0	-	3	108	107	99.1	-
K	0	0	0	-	3	111	111	100.0	-
Unweighted Means [6]									
Overall				95.2				98.4	96.9
Without G				97.6				99.4	98.9
Weighted Means [5]									
Overall				96.7				99.0	97.9
Without G				98.2				99.8	99.0

[1] Repeats - duplicate samples sent to the laboratories without their knowledge.

[2] Samples from the same animal included in two different trials.

[3] Samples from the same animal included twice in the same trial.

[4] Opportunity for agreement occurred whenever a sample was tested twice with the same reagent. (Reagents considered by a laboratory to be experimental were not included).

[5] Overall number of agreements as a percentage of overall number of possible agreements.

[6] Mean of laboratory means.

TABLE 3

AGREEMENT AMONG LABORATORIES BY REAGENT[1]

Reagent	Number of trials[2]	Per Cent of agreement: All laboratories — Number of observations[3]	All laboratories — Per Cent[4]	Excluding the laboratory causing most disagreement — Number of observations[3]	Excluding the laboratory causing most disagreement — Per Cent[4]
P	5	35	97.6	33	100.0
Z'	6	29	100.0	29	100.0
$\overline{FF}$	3	6	100.0	6	100.0
Y'(NF4;E_1)	2	11	100.0	11	100.0
O'(NF8;E_2)	2	9	100.0	9	100.0
I_2	1	3	100.0	3	100.0
B'(NF6;N_2)	1	4	100.0	4	100.0
A'_2	1	3	62.5	2	100.0
F	6	40	92.0	36	99.7
E	4	17	98.2	16	99.7
R	6	40	93.5	37	99.6
V	6	41	94.5	39	99.6
Q	5	29	85.7	27	99.5
I'	5	35	57.2	30	99.5
E'_2	5	21	92.6	18	99.2
A(A_2)	6	41	89.6	36	99.1
H	6	16	87.7	12	98.8
U_1	6	28	84.6	24	98.6
Y_2	6	39	91.9	35	98.6

TABLE 3 (continued)

K'	6	27	93.1	23	98.5
L	6	41	94.6	35	98.4
W	6	41	92.7	36	98.1
$\overline{ZZ}$	5	25	89.6	22	98.0
T_1	6	32	94.1	29	97.8
L'	5	24	85.4	21	97.6
J'	5	30	92.7	25	97.5
T_2	6	21	88.1	19	97.4
S	6	40	88.2	36	97.2
K	6	36	72.4	31	97.0
M	5	32	88.2	29	96.4
X_1	5	34	70.4	29	95.9
D'	6	40	86.3	36	95.8
C_2	6	35	88.4	30	95.1
Z	6	41	75.5	36	94.9
H'	6	38	63.9	33	94.7
G	6	41	82.9	36	94.6
B_2	4	10	82.8	8	93.8
U'	1	2	100.0	2	100.0
C_1	6	31	72.0	27	91.8
E'_3	5	32	83.1	28	91.0
A'	5	32	79.5	27	90.3
X_2	5	33	78.2	29	90.2

TABLE 3 (continued)

A_1	5	13	90.6	10	94.5
I	6	41	69.1	35	89.8
B	6	40	62.3	35	89.7
U_2	6	37	76.0	32	87.9
E'_1	6	38	57.7	33	87.0
O_3	5	33	71.7	28	85.9
O_1	6	36	63.1	31	85.5
O_x	1	2	85.0	2	85.0
Y_1	6	28	54.3	24	84.6
X_3	5	15	70.2	11	84.1
O_2	1	2	82.5	2	82.5
J	6	41	57.1	35	73.2
W_2	1	2	70.0	2	70.0
		1493		1315	
Overall Mean			82.1		94.6

1/ Arranged in decreasing order of agreement as determined when the laboratory causing the most disagreement is excluded.

2/ Number of trials in which at least two laboratories used the reagent.

3/ The number of laboratories using the reagent in each trial summed over-all trials.

4/ Weighted means - In calculating the overall mean each trial mean is weighted according to the number of laboratories using the reagent in that trial.

TABLE 4

AGREEMENT AMONG LABORATORIES BY BLOOD-GROUP SYSTEMS

System	Number of Reagents	Per Cent of agreement: All Laboratories — Number of Observations	All Laboratories — Per Cent	Excluding the laboratory causing most disagreement — Number of Observations	Excluding the laboratory causing most disagreement — Per Cent
FV	3	87	93.7	81	99.7
A	5	99	93.8	88	98.9
L	1	41	94.6	35	98.4
M	1	32	88.2	29	96.4
Z	2	66	80.8	58	96.1
C	10	272	83.6	238	95.2
SU	5	145	78.1	127	94.4
B	28	710	78.8	624	94.0
J	1	41	57.1	35	73.2